REMARKS

Claims 1-11 remain in the application. Claims 5 and 7 have been amended. A version with markings to show changes made follows page 13. Claims 12-15 have been added. The specification has been amended. Reconsideration of this application, as amended, is respectfully requested.

The specification has been amended to remove drawings therefrom. The drawings have been inserted into the appropriate location of the application.

Claim 5 has been amended to require the use of the electrode strip recited in claim 1.

Claim 7 has been amended to show that it depends from claim 6.

Claims 12 and 14 have been added to recite the species 1,7-phenanthroline quinone. Claims 13 and 15 have been added to recite the species 4,7-phenanthroline quinone. Support for these claims can be found at page 5, lines 28-30, page 6, lines 18-19, and page 7, lines 4-5 of the specification, and at page 25, TABLE 3 of the specification.

The specification was objected to because figures were incorporated into the body of the specification. This objection has been addressed by deleting the figures from the specification and incorporating them into the application as drawings. The specification has been amended to include a description of these drawings in the Brief Description of Drawings section of the application.

Claim 7 was objected to because the dependency of claim 7 was illegible. This objection has been addressed by the amendment to claim 7.

Claims 1-3 were rejected under 35 U. S. C. § 103(a) as being unpatentable over Geng et al., "Amperometric biosensors based on dehydrogenase/NAD and heterocyclic quinones", *Biosensors & Bioelectronics*, Vol. 11, No. 12, pp. 1267-1275, 1996, in view of MacFarlane et al. (USP 5,212,622) and Carter et al. (USP 5,628,890). This rejection is respectfully traversed for the following reasons.

Geng et al., "Amperometric biosensors based on dehydrogenase/NAD and heterocyclic quinones", *Biosensors & Bioelectronics*, Vol. 11, No. 12, pp. 1267-1275, 1996, (hereinafter "Geng et al."), discloses the electrocatalytic oxidation of NADH with heterocyclic quinones dissolved in a water solution. The heterocyclic

quinones used were 1,7-phenanthroline-5,6-dione and 1,10-phenanthroline-5,6-dione.

MacFarlane et al., U.S. Patent No. 5,212,622 (hereinafter "MacFarlane et al."), discloses a high surface area electrode comprising a composite of an electronically conductive particulate filler dispensed in a binder material, wherein the particles are substantially in intimate contact, and the binder material is able to support conduction of ions to and from a substantial proportion of the surface of the filler particles. The binder material may comprise a polymeric material having ionized groups and corresponding counterions.

Carter et al., U. S. Patent No. 5,628,890 (hereinafter "Carter et al."), discloses an electrode strip for use in an electrochemical sensor for measuring a compound in a sample, including an electrode support, a reference or counter electrode disposed on the support, a working electrode spaced from the reference or counter electrode on the support, a covering layer defining an enclosed space over the reference and working electrodes and having an aperture for receiving a sample into the enclosed space, and a plurality of mesh layers interposed in the enclosed space between the covering layer and the support, the covering layer having a sample application aperture spaced from said electrodes and said reference electrode spaced from said working electrode at a position remote from and on the opposite side of said working electrode from said aperture. The working electrode includes an enzyme capable of catalyzing a reaction involving a substrate for the enzyme or a substrate catalytically reactive with an enzyme and a mediator capable of transferring electrons transferred between the enzyme-catalyzed reaction and the working electrode to create a current representative of the activity of the enzyme and representative of the compound.

The electrode strip of the present invention requires an active electrode formulated with filler and binder ingredients, such that the electrode gives a monotonic response to concentrations of analyte between about 1 and 8 mM when measurement is made in a kinetic mode in which simultaneous oxidation and reduction of the mediator occurs during the measurement.

Geng et al. does not disclose or suggest the use of a binder. PEO is a polymer, but PEO does not function as a binder in an electrode strip. The binder

is employed in order to render the active electrode stable during the assay. The data in the present application show that Geng et al. does not disclose or suggest that the composition described therein will retain stability during the assay. See FIG. 10 of the present application (GENG PAPER), which shows that no peak current is observed after 60 seconds exposure. In contrast, FIG. 9 of the present application (PRESENT INVENTION) shows that a peak current is observed after 60 seconds exposure.

MacFarlane et al. does not disclose or suggest the use of binders with mediators. MacFarlane et al. is concerned with ion transfer. Thus, the problem addressed in MacFarlane et al. is not the same problem as that addressed by the present invention. In contrast to MacFarlane et al., Geng et al. is concerned with mediators, not ion transfer. Accordingly, it can be concluded that one of ordinary skill in the art would not be influenced to use the teachings of MacFarlane et al., which relate to ion transfer, to address the shortcomings in Geng et al., which relate to instability of compositions containing mediators. Furthermore, if one of ordinary skill in the art were to attempt to combine MacFarlane et al. with Geng et al., he would have had no way of knowing that such a combination would have provided the unexpected benefit of stability during the assay. In other words, at most, one of ordinary skill in the art could say that it would be obvious to try to combine MacFarlane et al. with Geng et al. However, "obvious to try" is not the test for obviousness under 35 U.S.C. § 103. According to In re Tomlinson, Hall, and Geigle, 150 USPQ 623 (CCPA 1966), at 626:

> ..... obviousness under section 103 is of *compositions* and *methods*, not of the direction to be taken in making *efforts* or *attempts*. Slight suggestion reflects, we think, that there is usually an element of "obviousness to try" in any research endeavor, that it is not undertaken with complete blindness but rather with some semblance of a chance of success, and that patentability determinations based on that as the test would not only be contrary to statute but result in a marked deterioration of the entire patent system as an incentive to invest in those efforts and attempts which go by the name of "research."

disagree MacFarlane drawn to keeping ionic material in electrodes from leaching out Analogous to current problem

I never said obvious to try

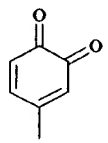
Neither Geng et al. not MacFarlane et al. makes any statement that would lead one to believe that the combination of these two references would result in enhancing the stability of a phenanthroline quinone during an assay for glucose.

Carter et al. merely discloses the use of a mediator in general. Carter et al. does not disclose or suggest the use of the specific class of mediators recited in the claims of this application. In view of the foregoing, it is submitted that the combination of Geng et al., MacFarlane et al., and Carter et al. does not render the claims of this application obvious to one of ordinary skill in the art.

Claims 1-4 were rejected under 35 U. S. C. § 103(a) as being unpatentable over Batchelor et al., "AMPEROMETRIC ASSAY FOR THE KETONE BODY 3-HYDROXYBUTYRATE", *Analytica Chimica Acta*, 221 (1989) 289-294, in view of Geng et al., MacFarlane et al., and Carter et al. This rejection is respectfully traversed for the following reasons.

Batchelor et al., "AMPEROMETRIC ASSAY FOR THE KETONE BODY 3-HYDROXYBUTYRATE", *Analytica Chimica Acta*, 221 (1989) 289-294 (hereinafter "Batchelor et al."), discloses a dry-strip electrochemical sensor for the direct measurement of 3-hydroxybutyrate in blood. The sensor utilizes the electrocatalytic oxidation of enzymically generated NADH by the redox mediator 4-methyl-o-quinone. The enzyme 3-hydroxybutyrate dehydrogenase, cofactor NAD^+ and 4-methyl-o-quinone were incorporated into single-use disposable strip electrodes.

Batchelor refers to the use of 4-methyl-o-quinone (4-methyl-1,2-benzoquinone) as a mediator for NADH in the construction of a biosensor electrode for D-3-hydroxybutyrate, which is a ketone. The structure of 4-methyl-o-quinone is:



This mediator is not desired for use in electrodes designed to determine the concentration of D-3-hydroxybutrate and glucose.

First, 4-methyl-o-quinone is an unstable compound and must be stored in the dark at -20°C. 4-Methyl-o-quinone is very reactive; it dimerizes readily, i.e., it reacts with itself. See the data regarding 4-methyl-1,2-benzoquinone from the Combined Chemical Dictionary, attached hereto and marked as Exhibit IA. The dimerization reaction will cause problems in handling during the fabrication of electrode strips and will result in an inadequate shelf life of the electrode strip when stored at ambient temperature.

not sure of point of this I'm not relying on 4-MQ for rej.

Second, 4-methyl-o-quinone inhibits NAD-dependent enzymes, such as D-3-hydroxybutyrate dehydrogenase and glucose dehydrogenase, which are used in the fabrication of electrodes designed to determine the concentration of D-3-hydroxybutyrate and glucose, respectively. The data in EXAMPLE 1 and TABLE 3 of the present application show that 96% of D-3-hydroxybutyrate dehydrogenase activity is lost in the presence of 4-methyl-o-quinone, while only 4% of D-3-hydroxybutyrate dehydrogenase activity is lost in the presence 1,10-phenanthroline quinone. The disadvantage relating to inhibition of NAD-dependent enzymes could possibly be addressed by using excess amounts of NAD-dependent enzymes. However, because NAD-dependent enzymes are expensive, the use of excess amounts of NAD-dependent enzymes would make the electrode strip prohibitively expensive.

One of ordinary skill in the art would not have been influenced by the teachings of Geng et al. to address the shortcomings of the 4-methyl-o-quinone described in Batchelor et al. for a variety of reasons. One of ordinary skill in the art would not have known from the teachings of Geng et al. that 1,10-phenanthroline quinone and 1,7- phenanthroline quinone do not inhibit NAD-dependent hydrogenase enzymes, such as glucose dehydrogenase. Second, one of ordinary skill in the art would not have known from the teachings of Geng et al. that the use of a phenanthroline quinone mediator provides a longer shelf-life and allows the use of smaller amounts of expensive enzyme.

whether this is true or not doesn't obviate that mediators of Geng found utility in other NAD applications

Furthermore, Applicants discovered that 1,10- phenanthroline quinone is a relatively inefficient NADH-mediator (see EXAMPLE 1, TABLE 3, page 25, line 8 through page 26, line 8 of the specification and EXAMPLE 2, Evaluation of Meldola's Blue and 1,10- PQ in Dry Strips containing HBDH). One of ordinary skill in the art would not have known from the teachings of Geng et al. that a

relatively inefficient mediator would provide such unexpected benefits as a mediator. The extent of the unexpected benefits can be described as follows.

These benefits do not obviate a rejection

The response of the electrode strip of the present invention involves at least two components. The first component involves the rate of reaction of the substrate (e.g., glucose, hydroxybutyrate) with the NAD-dependent dehydrogenase enzyme (e.g., glucose dehydrogenase, D-3-hydroxybutyrate dehydrogenase). This reaction generates NADH from NAD. The more NADH that can be produced in the reaction, the greater the possible response of the electrode strip. The rate of formation of NADH should be constant for different electrodes systems for a given value of enzyme activity. However, mediators such as Meldola's Blue and 4-methyl-o-quinone destroy enzyme activity so much that less NADH will be generated by these mediators in comparison with the amount of NADH generated when mediators of the present invention, such as 1,10- phenanthroline quinone, 1,7- phenanthroline quinone and 4,7- phenanthroline quinone, are used. This destruction of enzyme activity will also shorten the shelf life of the electrode strip, because the least stable active component selected from the mediator, enzyme, and NAD of the formulation of the working electrode is the enzyme. Applicants have shown that the mediators of the present invention do not destroy enzyme activity. EXAMPLE 1 of the application, page 25, TABLE 3 shows that six times more enzyme activity remains for 1,10- phenanthroline quinone as compared with Meldola's Blue.

The second component involves the rate of reaction of the mediator with NADH. In the present application, the mediators are reduced by NADH and then re-oxidized at the electrode to generate a measurable current. It is preferred that a mediator should react as rapidly as possible with NADH to maximize this component of the electrode response. Meldola's Blue reacts much faster with NADH than does the mediator of the present invention, e.g., 1,10-phenanthroline quinone. See EXAMPLE 1 of this application, page 24, lines 9-14. FIGS. 3 and 4 show that Meldola's Blue reacts with NADH fifteen times faster than does 1,10-phenanthroline quinone (slope for Meldola's Blue = 84.8 μA/mM and slope for 1,10- phenanthroline quinone = 5.8 μA/mM for the NADH calibration of the two systems). Prior to the discoveries by Applicants, it would not have been obvious to one of ordinary skill in the art to choose 1,10- phenanthroline quinone over

explain Geng then.

Meldola's Blue as an NADH mediator for use in an electrode strip.

In summary, although 1,10- phenanthroline quinone is a much slower mediator for NADH than Meldola's Blue, 1,10- phenanthroline quinone does not destroy enzyme activity as much as does Meldola's Blue. Therefore, it would not have been obvious to one of ordinary skill in the art to use 1,10-phenanthroline quinone and related compounds in place of 4-methyl-o-quinone or any other of the known NADH mediators to produce a commercially viable electrode strip. In view of the foregoing, it is submitted that the combination of Batchelor et al., Geng et al., MacFarlane et al., and Carter et al. does not render the claims of this application obvious to one of ordinary skill in the art.

Claim 4 was rejected under 35 U. S. C. § 103(a) as being unpatentable over Geng et al., MacFarlane et al., and Carter et al. and further in view of Batchelor et al. This rejection is respectfully traversed for the following reasons.

The reasons for the traversal of this ground of rejection are the same as those discussed on pages 7 through 10 of this AMENDMENT AND RESPONSE with respect to the previous ground of rejection, in which the references Batchelor et al., Geng et al., MacFarlane et al., and Carter et al. were cited.

Claims 5 and 8-11 were rejected under 35 U. S. C. § 103(a) as being unpatentable over Batchelor et al. in view of Geng et al. This rejection is respectfully traversed for the following reasons.

Claim 5 has been amended to recite that the method requires the electrode strip of claim 1. As stated previously, with respect to the rejections of claims 1-4, one of ordinary skill in the art would not have been influenced by the teachings of Geng et al. to address the shortcomings of the 4-methyl-o-quinone described in Batchelor et al. One of ordinary skill in the art would not have known from the teachings of Geng et al. that 1,10- phenanthroline quinone and 1,7-phenanthroline quinone do not inhibit NAD-dependent hydrogenase enzymes, such as glucose dehydrogenase. One of ordinary skill in the art would not have known from the teachings of Geng et al. that the use of a phenanthroline quinone mediator provides a longer shelf-life and allows the use of smaller amounts of expensive enzyme. Furthermore, although 1,10- phenanthroline quinone is a much slower mediator for NADH than Meldola's Blue, 1,10- phenanthroline quinone does not destroy enzyme activity as much as does Meldola's Blue.

Therefore, it would not have been obvious to one of ordinary skill in the art to use 1,10-phenanthroline quinone and related compounds in place of 4-methyl-o-quinone or any other of the known NADH mediators to produce a commercially viable electrode strip. For the foregoing reasons, the combination of Batchelor et al. and Geng et al. does not render the claims of this application obvious to one of ordinary skill in the art.

Claims 5 and 8-11 were rejected under 35 U. S. C. § 102(b) as anticipated by or, in the alternative, under 35 U. S. C. § 103(a) as obvious Geng et al. in view of Batchelor et al. This rejection is respectfully traversed for the following reasons.

Claim 5 has been amended to recite that the method requires the electrode strip of claim 1. As stated previously, with respect to the rejections of claims 1-4, one of ordinary skill in the art would not have been influenced by the teachings of Geng et al. to address the shortcomings of the 4-methyl-o-quinone described in Batchelor et al. One of ordinary skill in the art would not have known from the teachings of Geng et al. that 1,10- phenanthroline quinone and 1,7-phenanthroline quinone do not inhibit NAD-dependent hydrogenase enzymes, such as glucose dehydrogenase. One of ordinary skill in the art would not have known from the teachings of Geng et al. that the use of a phenanthroline quinone mediator provides a longer shelf-life and allows the use of smaller amounts of expensive enzyme. Furthermore, although 1,10- phenanthroline quinone is a much slower mediator for NADH than Meldola's Blue, 1,10- phenanthroline quinone does not destroy enzyme activity as much as does Meldola's Blue. Therefore, it would not have been obvious to one of ordinary skill in the art to use 1,10-phenanthroline quinone and related compounds in place of 4-methyl-o-quinone or any other of the known NADH mediators to produce a commercially viable electrode strip. For the foregoing reasons, the combination of Geng et al. and Batchelor et al. does not render the claims of this application obvious to one of ordinary skill in the art.

Claims 6 and 7 were rejected under 35 U. S. C. § 103(a) as being unpatentable over Geng et al. and Batchelor et al. and further in view of MacFarlane et al. This rejection is respectfully traversed for the following reasons.

Claim 6 depends from claim 5. Claim 7 depends from claim 6. Claim 5 has been amended to recite that the method requires the electrode strip of claim 1. As stated previously, MacFarlane et al. does not disclose or suggest the use of binders with mediators. MacFarlane et al. is concerned with ion transfer. Thus, the problem addressed in MacFarlane et al. is not the same problem as that addressed by the present invention. In contrast to MacFarlane et al., Geng et al. is concerned with mediators, not ion transfer. Accordingly, it can be concluded that one of ordinary skill in the art would not be influenced to use the teachings of MacFarlane et al., which relate to ion transfer, to address the shortcomings in Geng et al., which relate to instability of compositions containing mediators. Furthermore, if one of ordinary skill in the art were to attempt to combine MacFarlane et al. with Geng et al., he would have had no way of knowing that such a combination would have provided the unexpected benefit of stability during the assay. In other words, at most, one of ordinary skill in the art could say that it would be obvious to try to combine MacFarlane et al. with Geng et al. However, "obvious to try" is not the test for obviousness under 35 U.S.C. § 103.

As stated previously, one of ordinary skill in the art would not have been influenced by the teachings of Geng et al. to address the shortcomings of the 4-methyl-o-quinone described in Batchelor et al. for a variety of reasons. One of ordinary skill in the art would not have known from the teachings of Geng et al. that 1,10- phenanthroline quinone and 1,7- phenanthroline quinone do not inhibit NAD-dependent hydrogenase enzymes, such as glucose dehydrogenase. Second, one of ordinary skill in the art would not have known from the teachings of Geng et al. that the use of a phenanthroline quinone mediator provides a longer shelf-life and allows the use of smaller amounts of expensive enzyme. Furthermore, although 1,10- phenanthroline quinone is a much slower mediator for NADH than Meldola's Blue, 1,10- phenanthroline quinone does not destroy enzyme activity as much as does Meldola's Blue. Therefore, it would not have been obvious to one of ordinary skill in the art to use 1,10-phenanthroline quinone and related compounds in place of 4-methyl-o-quinone or any other of the known NADH mediators to produce a commercially viable electrode strip. For the foregoing reasons, the combination of Geng et al., Batchelor et al., and

MacFarlane et al. does not render the claims of this application obvious to one of ordinary skill in the art.

In view of the foregoing, it is submitted that claims 1-15 are in condition for allowance, and official Notice of Allowance is respectfully requested.

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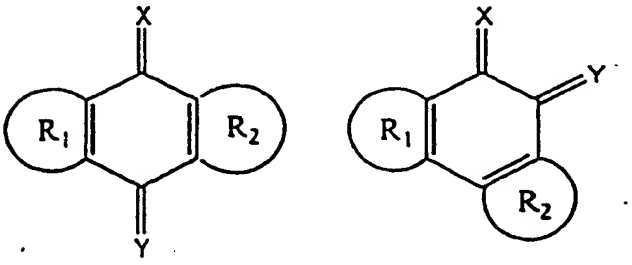
VERSION WITH MARKING S TO SHOW CHANGES MADE

5. (Once amended) A process of measuring the concentration in an aqueous sample of an analyte subject to oxidation by a $NAD(P)^+$ dependent enzyme comprising the steps of:

a) providing the electrode strip of claim 1;

[a)] b) oxidizing the analyte with the $NAD(P)^+$ dependent enzyme in the presence of $NAD(P)^+$;
oxidizing the NAD(P)H generated by reaction with the analyte and $NAD(P)^+$ dependent enzyme with [a] the mediator compound of step a) [having one of the following two formulae:



where X and Y can independently be oxygen, sulphur, CR^3R^4, NR^3, or 10 NR^3R^4 or the functional group CZ^1CZ^2, where Z^1 and Z^2 are electron withdrawing groups; R_1 and R_2 can independently be a substituted or unsubstituted aromatic or heteroaromatic group; and R^3 and R^4 can independently be a hydrogen atom, a hydroxyl group or a substituted or unsubstituted alkyl, aryl, heteroaryl, amino, alkoxyl, or aryloxyl group];
and

[b)] c) applying an electrical potential at an electrode to reoxidize the mediator compound reduced in oxidizing NAD(P)H and observing the resultant current,
wherein som of the mediator compound is being reduced by reaction with

NAD(P)H while some of the mediator compound is being oxidized by transfer of electrons to said electrode during a measurement period and the rate of oxidation of the mediator compound over said measurement period and consequently the resultant observed current is monotonically related to the concentration of analyte in the sample.

7. (Once amended) The process of claim [6] 6 wherein the current observed during the measurement period is linearly related to the concentration of analyte in the sample.

DATABASE

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NAME	4-Methyl-1,2-benzoquinone
	View Structure View Diagram
CAS NUMBER	3131-54-2
SYNONYM(S)	4-Methyl-3,5-cyclohexadiene-1,2-dione 4-*o*-Toluquinone
MOLECULAR FORMULA	$C_7H_6O_2$
MOLECULAR WEIGHT	122.123
STATE CHANGE	Mp 68°
PHYSICAL DESCRIPTION	Red needles (Et_2O)
MISC. INFORMATION	Readily dimerises
REFERENCES	Willstätter, R. *et al*, *Ber.*, 1911, **44**, 2171 (synth) Teuber, H.-J. *et al*, *Chem. Ber.*, 1955, **88**, 802 (synth, ir) Otting, W. *et al*, *Chem. Ber.*, 1955, **88**, 828 (ir) Thomson, R.H., *Naturally Occurring Quinones*, Academic Press, London, 1971, Hollenstein, R. *et al*, *Helv. Chim. Acta*, 1973, **56**, 320 (cmr, pmr) Durst, H.D. *et al*, *J.O.C.*, 1975, **40**, 268 (synth)

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